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SEQUENCE UPDATE

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Promoter region of *Ory s 1*, the major rice pollen allergen gene

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Grass pollen allergy afflicts up to 25% of the population in cool temperate climates. IgE-mediated symptoms such as rhinitis and bronchial asthma are triggered by the release of proteins when grass pollen grains contact the moist surface of the human respiratory tract. Allergenic proteins of grass pollen have been classified into different groups according to their physiochemical and immunological properties (Marsh 1975). The major and most widespread allergenic components belong to group 1. Group 1 allergens are glycoproteins of 32–35 kDa which are rapidly released when pollen is in contact with moist surfaces (Marsh 1975; Singh et al 1990). Group 1 allergens are named after the source species, e.g. Lol p 1 is from *Lolium perenne* (ryegrass), Zea m 1 from *Zea mays* (maize) and Ory s 1 from *Oryza sativa* (rice) (Marsh et al. 1988).

We previously reported molecular cloning and immunological characterisation of group 1 allergens from various grass genera including ryegrass (Griffith et al. 1991), Bermuda grass (Smith et al. 1994) and rice (Xu et al. 1995). High levels of sequence conservation were found among homologues in divergent grass species, suggesting that these glycoproteins may have a conserved biological function. Shcherban et al. (1995) reported that group 1 pollen allergens have a distant sequence similarity to expansins, the extracellular proteins that promote cell wall enlargement. Recently, Cosgrove et al. (1997) provided strong evidence for expansin-like activity of the maize group 1 allergen. It was further proposed that group 1 allergens facilitate pollen tube growth through maternal tissues by loosening the cell walls of grass stigma and style. Owing to the biological significance of group 1 pollen allergens, we are interested in the functional analysis of the promoter region of the genes encoding these proteins.

For isolation of the *Ory s 1* promoter, a rice (*Oryza sativa* L. Japonica) genomic library purchased from Clontech Laboratory was screened using an *Ory s 1* cDNA as a probe. Out of approximately 60 000 recombinant clones, one positive clone was obtained. This clone was subjected to restriction mapping and Southern blot analysis. A 4.0-kb fragment showing hybridisation to the cDNA probe in a DNA gel blot was partially sequenced.

A total of 2907 bp of DNA sequence was determined, which included 1523 bp of 5′ upstream region, the entire transcriptional region and 198 bp of 3′ flanking region. No intron was located in the genomic clone. A putative TATA box sequence (TAAATA) is located at position 1485 bp. Searches in databases with the 5′ upstream region of *Ory s 1* did not reveal any common sequence elements with other known pollen-specific genes. The translational start of the *Ory s 1* gene is the ATG codon present at position 1547 bp. This start codon gives the longest possible open reading frame of 801 bp and encodes 267 amino acids. Comparison of the corresponding region of *Ory s 1* cDNA revealed an additional four residues at amino acid position 135. This suggests that the isolated genomic clone may correspond to another member of the multigene family. A database search revealed that the coding region of *Ory s 1* shared high levels of sequence identity (62–65%) with group 1 allergens of other grasses.

To determine the activity of the isolated *Ory s 1* promoter, we made a translational fusion between the 1507-bp 5′ upstream region of *Ory s 1* with the *GUS* reporter gene in the binary vector pBI101 (Jefferson et al. 1987). This *Ory s 1-GUS* chimeric gene was used for transient expression in mature pollen of *Nicotiana tabacum* and *N. alata* by microprojectile bombardment as described by

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Twell et al. (1991a). Histochemical staining showed that following microprojectile bombardment the *GUS* gene directed by the *Ory s 1* promoter was expressed at high levels in mature pollen and germinated pollen of *Nicotiana*. The activity of the *Ory s 1-GUS* chimeric gene was further tested in freshly dissected rice anthers following microprojectile bombardment. GUS activity was detected in the bombarded pollen but not in control pollen.

Activity of the *Ory s 1* promoter was further examined in stably transformed tobacco plants using *Agrobacterium*-mediated transformation as described previously (Xu et al. 1993). A total of eighteen primary transformants with stable integration of the *Ory s 1-GUS* gene fusion were regenerated. DNA gel blot analysis confirmed that all transgenic plants carried either one or two insertions of the transgenes. Histochemical staining showed that 14 plants exhibited high levels of GUS activity in mature pollen. In most cases, blue staining was visible after 1 h incubation in X-Gluc substrate. The quantity of blue-stained pollen in these plants ranged from 50–100%. GUS activity was also examined at early stages of pollen development. In the unicellular microspore stage, no detectable GUS staining was observed. Weak blue staining was visible in pollen at the early bicellular stage and the intensity increased sharply in mature pollen. No GUS activity was detectable in other vegetative or floral tissues of these transformed plants.

In addition GUS enzyme activity in various tissues and pollen at different developmental stages of individual transformants T14 and T16 (with 100% blue-stained pollen in histochemical assay) was measured quantitatively using fluorometric GUS assays as described by Jefferson (1987). GUS activity as measured fluorimetrically was undetectable in vegetative and floral tissues. GUS activity was, however, detectable in unicellular microspores of both transgenic plants. Mature pollen of both plants (T14 and T16) exhibited a very high level (12- to 15-fold) of GUS enzyme activity compared to the background activity in pollen of untransformed control plants. Our results show that *GUS* gene expression in transgenic *Nicotiana* plants was activated in the early bicellular pollen stage and enhanced dramatically during pollen maturation. The pattern of *Ory s 1-GUS* gene expression in tobacco corresponded to that of endogenous *Ory s 1* in rice (Xu et al. 1995). These results indicate that the isolated *Ory s 1* promoter is able to direct correct gene expression in heterologous tobacco plants. It is interesting to note that the *Ory s 1* promoter is highly active in pollen of both monocots and dicots. Similar results have been reported in *Zmg13*, a pollen promoter from *Zea mays* (Guerrero et al. 1990; Hamilton et al. 1992). In contrast, dicot pollen promoters have been reported to be either inactive or expressed poorly in monocots (Twell et al. 1991b; Leede-Plegt et al. 1992). This implies that gene expression in monocots may require unique regulatory factors. In conclusion, the use of a reporter gene (*GUS*) fused to the *Ory s 1* promoter has enabled us to demonstrate that genes encoding group 1 allergens are specifically expressed in pollen. The fact that the activity of the *Ory s 1* promoter is conserved in both monocots and dicots makes it an ideal tool for directing pollen specific gene expression in transgenic plants.

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